Original article

The role of miR-17-1-3p in mitochondrial fusion gene expressions and muscle biogenesis with swimming exercise intervention in metabolic syndrome rat model

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Abstract. *Background and aim:* it is known that exercise regulates the expression of mitochondrial fusion and muscle biogenesis involved genes. However, the mechanisms underlying the changes in mitochondrial fusion and muscle metabolism caused by exercise have not been clearly revealed yet. The aim of the study is to elucidate the molecular mechanisms of exercise therapy in metabolic syndrome. *Methods:* 21 Sprague-Dawley male rats were used in the study and by adding %30 fructose into drinking water Metabolic Syndrome (Mets) were induced after 5 weeks. The rats were randomly divided into 3 groups (Control, MetS, MetS+Exercise) as 7 rats in each cage after feeding for 5 weeks with 30% fructose diet. Swimming exercise were applied to the MetS+Exercise group for six weeks. Once the interventions were finished, the rats were decapitated and dissected to separate the skeletal muscle tissue samples. For the detection of alterations in the expression levels of MFN1, MFN2 and MSTN genes and miR-17-1-3p, the quantitative polymerase chain reaction (qPCR) was performed. *Results:* MFN1 and MFN2 expressions upregulated after exercise therapy in intervention group. MSTN and miR-17-1-3p were downregulated after exercise intervention. *Conclusions*: it has been shown in the present study, treating MetS with exercise therapy upregulates Mfn1 and Mfn2 gene expressions, which are involved in mitochondrial fusion dynamics, and that MSTN expression, which is increased in MetS, is downregulated by exercise intervention. To the best of our knowledge we demonstrated for the first time that exercise may regulates MSTN, Mfn1 and Mfn2 gene expressions by suppressing miR-17-1-3p.

Key words: Exercise, Metabolic syndrome, miR-17-1-3p, MFN1, MFN2, MSTN

Introduction

Mitochondria is an important organelle producing energy primarily through oxidative phosphorylation and plays a key role in calcium signaling, regulation and synthesis of cellular metabolism, steroid synthesis, and perhaps most importantly in apoptosis (1). Mitochondrial fusion and fission processes are necessary dynamics for the formation of the current form of mitochondria. The mitofusin 1(Mfn1), mitofusin 2(Mfn2) and Opa1 genes control the fusion dynamic (2), while the Drp1 gene controls the fission dynamic (1). Metabolic diseases can cause apoptosis in mitochondria by creating imbalances in mitochondrial dynamics.

Metabolic syndrome (MetS), a fatal endocrinopathy with a combination of systemic damage such as abdominal obesity, glucose intolerance, impaired lipid profile, hypertension and coronary artery disease triggered by insulin resistance (3), is associated with

mitochondrial dysfunction (4). MetS triggers the downregulation of Mfn1, Mfn2 and optic atrophy 1 Opa1 gene expressions, which control mitochondrial fusion dynamics (5,6).

Dysfunction of mitochondria in skeletal muscle cells, is associated with increased MSTN gene expression, whose upregulation is known to cause muscle atrophy (7). While MSTN, which has an anti-anabolic property, plays a major role in diseases such as cachexia and sarcopenia, in which skeletal muscle mass loss is experienced, its expression is known to increase due to oxidative stress in skeletal muscle cells (8). In a previous study, we showed that serum myostatin levels increased with MetS induced oxidative stress (9).

miRNAs are a class of small non-coding RNAs that regulate gene expression post-transcriptionally (10). They are considered stable in healthy individuals, but external factors, including lifestyle, can affect their expression (11-13). On the other hand, changes and/or abnormalities in some miRNA expression levels are associated with the occurrence of mitochondrial dysfunction and metabolic diseases, and cellular oxidative stress (14). Moreover, miRNAs are related to MetS because of the important role that they play in lipoprotein metabolism by targeting different protein-coding mRNA's (15). In addition, it is well-known that miRNAs play an important role in the regulation of the mitochondrial dynamics and the cell cycle (16). Some of the mir-17 family members (miR-17, miR-17-3p, miR-17-92) has been shown to be associated with cardiac reprogramming in response to aerobic exercise (17-21). On the other hand, the relationship of miR-17-1-3p with exercise, which has been shown to play a role in growth and development in some tissues or in the control of the nervous system (22-24) has not been revealed yet.

During the adaptation of skeletal muscles to exercise, improvements occur in molecular bases such as increased mitochondrial mass, altered substrate metabolism, enhanced angiogenesis or myofibril hypertrophy (25,26). In terms of its molecular effects, it can be said that exercise upregulates the expression of mitochondrial fusion genes (27,28). The mechanisms underlying the changes in mitochondrial fusion and muscle metabolism caused by exercise, which is an effective treatment method in metabolic diseases, have not been clearly revealed yet. With this study, for the first time we demonstrate the potential role of miR-17-1-3p, downregulated by exercise, in elucidating the molecular mechanism underlying mitochondrial dysfunction and skeletal muscle atrophy, which is likely to occur with MetS.

Methods

Experimental design

In this study 21 Sprague-Dawley male rats were used and by adding %30 fructose into drinking water Mets were induced after 5 weeks. (29) High serum glucose levels (>110 mg/dl), elevated serum triglyceride levels (>150 mg /dl), and low serum HDL-C levels (< 40 mg/dl) are considered to diagnose MetS. At the end of each week, weight controls were made. Control group was fed with standard diet and normal tap water. After 5 weeks of %30 fructose added drinking water diet, the rats (6-8 weeks old, weighing 200-250 g) were randomly divided into 3 groups as 7 rats in each cage (9).

Control group: In this group MetS was not induced and exercise was not applied.

MetS: MetS group without exercise application

MetS+Exercise: MetS group with exercise application

Exercise protocol

For swimming exercise interventions, A 30 cm deep and 184 cm wide water tank was used. Swimming exercise interventions applied for three days a week and for six weeks. Exercise interventions were applied for 20 minutes a day and between 09:00 and 10:00 in the morning. During the exercises, the rats were allowed to swim with their own body weight without any weight attached (9).

Sample collection

After all the interventions were finished, the rats were decapitated and dissected to separate the skeletal muscle tissue samples. Skeletal muscle specimens were

stored at –80°C after being frozen in liquid nitrogen until used for molecular analysis.

Quantitative polymerase chain reaction

The quantitative polymerase chain reaction (qPCR) was performed to evaluate the levels of MFN1, MFN2, MSTN and miR-17-1-3p exressions. For isolation of total RNA from rat skeletal muscle tissue samples Trizol solution were used (GeneAll Biotechnology Co., Ltd., Seoul, Korea) according to the recommended protocol. By using a micro spectrophotometer (Hangzhou Allsheng Instruments Co., Ltd., China) the obtained RNA samples were evaluated in terms of quantity and quality. After this, the RNA samples were converted to complementary DNA (cDNA) by using the OneScript cDNA synthesis kit (Applied Biological Materials Inc., Canada) in the thermal cycle device (Applied Biosystems, Singapore) according to the study previously indicated by Tektemur et al. 2021 (30). In the presence of sequence-specific primers, the obtained cDNA samples were amplified by the qPCR method using the EvaGreen 2X qPCR MasterMix-Low ROX (Applied Biological Materials Inc., Canada) in the ABI 7500 Real-Time PCR device (Applied Biosystems, Singapore). The qPCmethR protocol was set for 10 min at 95°C followed by 40 cycles 15 s at 95°C and 1 min at 60°C. Beta-actin (ACTB) was used as reference gene to normalization of the mRNA expression levels (Table 1). Also, to evaluate the microRNA expression level, small nucleolar RNA, C/D box 48 (SNORD48; RNU48) was used as a reference gene (Table 2). The 2–ΔΔCt method was used in the calculation of the differences between gene expressions.

Statistical evaluations

The analysis of the obtained data was carried out in the IBM-SPSS 22 program using the Mann-Whitney U and Kruskal-Wallis tests. $p < 0.05$ value was considered statistically significant.

Results

1- Exercise upregulates Mfn1 and Mfn2 mRNA expressions in skeletal muscle tissue

MetS caused a downregulation of both Mfn1 and Mfn2 mRNA expressions in skeletal muscle. But this downregulation was not significant. After the six weeks of swimming exercise interventions both Mfn1 and Mfn2 mRNA expressions upregulated significantly ($p<0.05$; Figure 1, Figure 2).

2- MetS-induced upregulation of MSTN mRNA expression was normalized by exercise in skeletal muscle tissue

MetS caused a significant upregulation in MSTN mRNA expression in skeletal muscle tissue ($p<0.05$). After the six weeks of swimming exercise interventions MSTN mRNA expression downregulated in

Table 1. Primer list of genes used for qPCR in the study.

Gene name	Symbol	Primer sequence (5'-3')		Size (bp)	Accession no
Actin beta*	ACTB	**F**	CCCATCTATGAGGGTTACGC	150	NM_031144.3
		R	TTTAATGTCACGCACGATTT		
Mitofusin 1	MFN1	**F**	TCAACGCTGATGAACACGGA	103	NM_138976.1
		R	AAGCAGAAGCATCCCAACGA		
Mitofusin 2	MFN2	**F**	TCAAGCGCCAGTTTGTGGAG	118	NM_130894.4
		R	CACAGATGAGCAAATGTCCCAGA		
Myostatin	MSTN	**F**	CAGACACACCCAAGAGGTCC	106	NM_019151.1
		R	AAGGCTTCGAAATCGACCGT		

*Used as reference gene for ion channel genes expression levels.
F: Forward primer
R: Reverse primer

Table 2. Primer list of miRNAs used for qPCR in the study.

Gene name	Symbol	Primer sequence (5'-3')	
Small Nucleolar RNA, C/D Box 48*	Snord48 (Rnu48)	**RT**	GTCTCCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGGAGACGGTCAG
		F	TCTGAGTGTCTTCGCTGACG
		R	GAGGTATTCGCACCAGAGGA
MicroRNA 17-1-3P	miR-17-1-3p	**RT**	GAAAGAAGGCGAGGAGCAGATCGAGGAAGAAGACGGAAGAATGTGCGTCTC GCCTTCTTTCCCACAAGT
		F	ACTGCAGTGAAGGCACTTGTGG
		uR	CGAGGAAGAAGACGGAAGAAT

*Used as reference gene for miRNAs expression levels.
RT: Reverse transcription primer for cDNA synthesis
F: Forward primer
R: Reverse primer
uR: Universal reverse primer

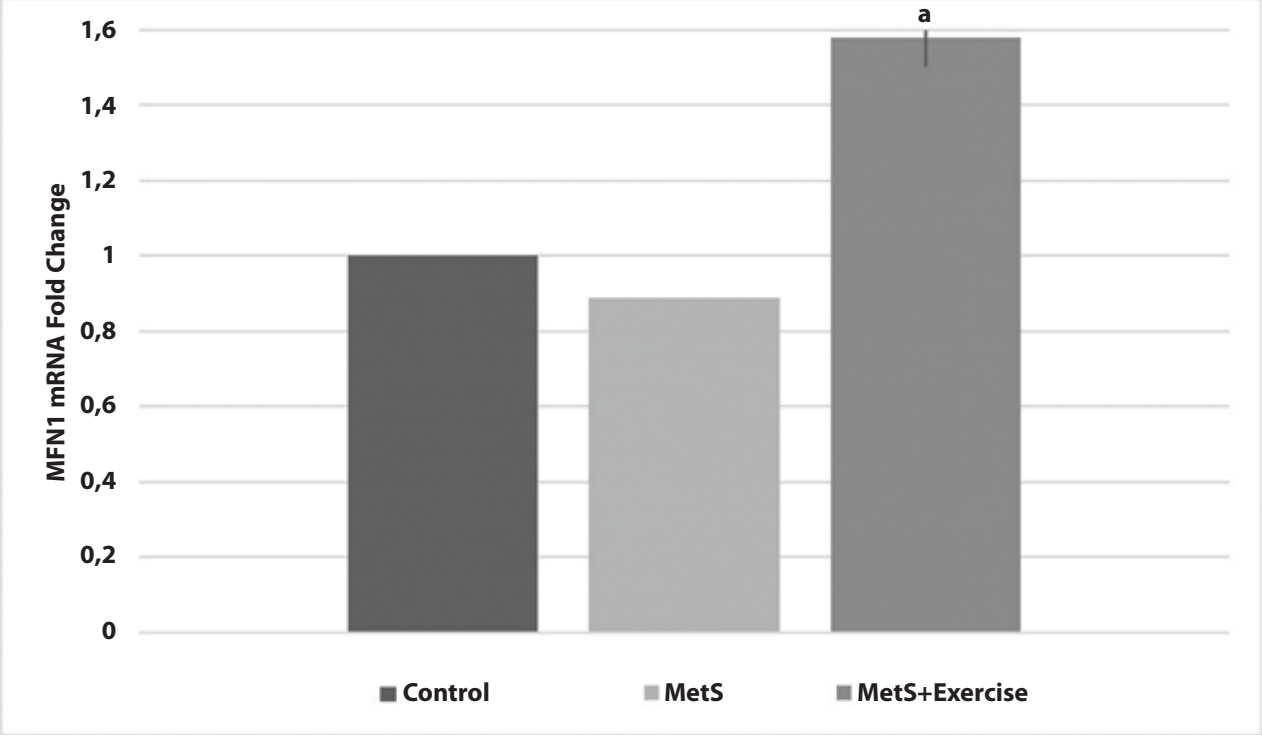

Figure 1. Mfn1 mRNA Fold Change.

exercise intervention group significantly to the level of control group ($p<0.05$; Figure 3).

3- Exercise suppressed the miR-17-1-3p expression in skeletal muscle tissue

There was no significant change in miR-17-1-3p change in MetS group compared to control group ($p>0.05$). However, after the six weeks of swimming exercise intervention it was seen that the miR-17-1-3p expression was downregulated significantly compared to MetS group ($p<0.05$; Figure 4).

Discussion and conclusion

Sedentary life style and eating habits with high fructose foods, which are widely used in the food

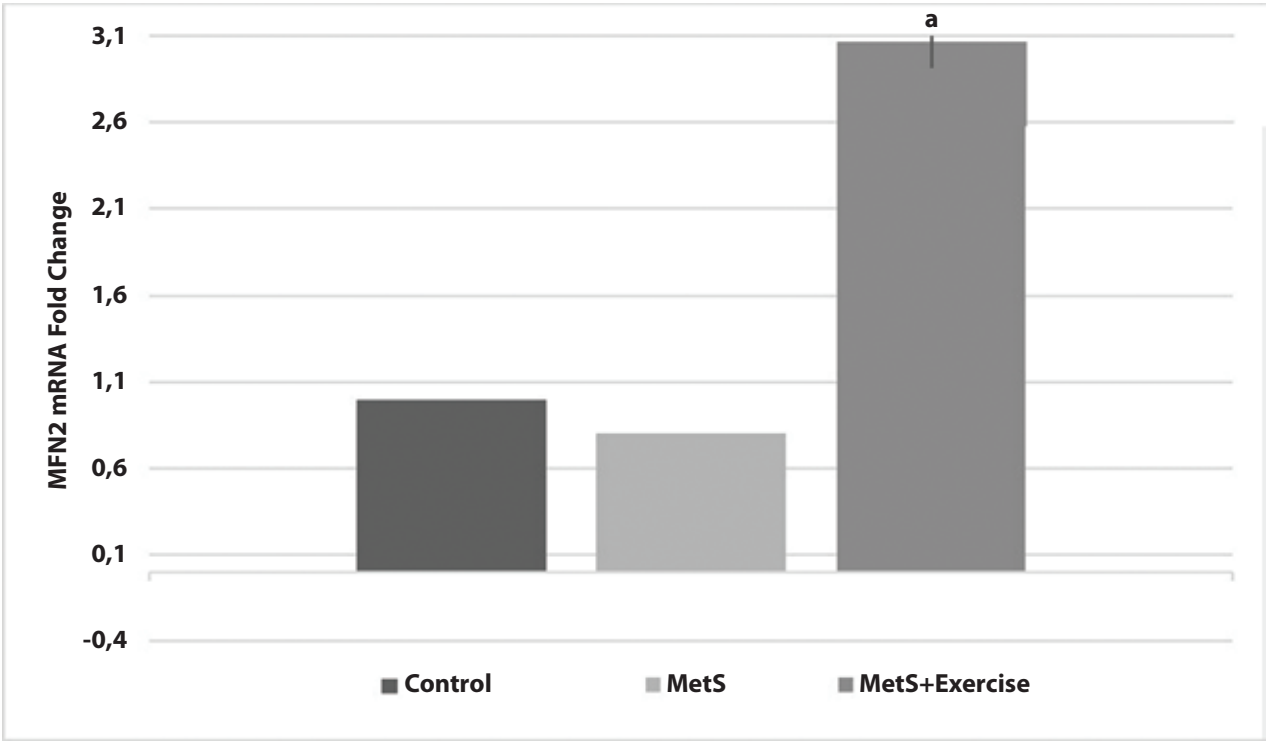

Figure 2. Mfn2 mRNA Fold Change.

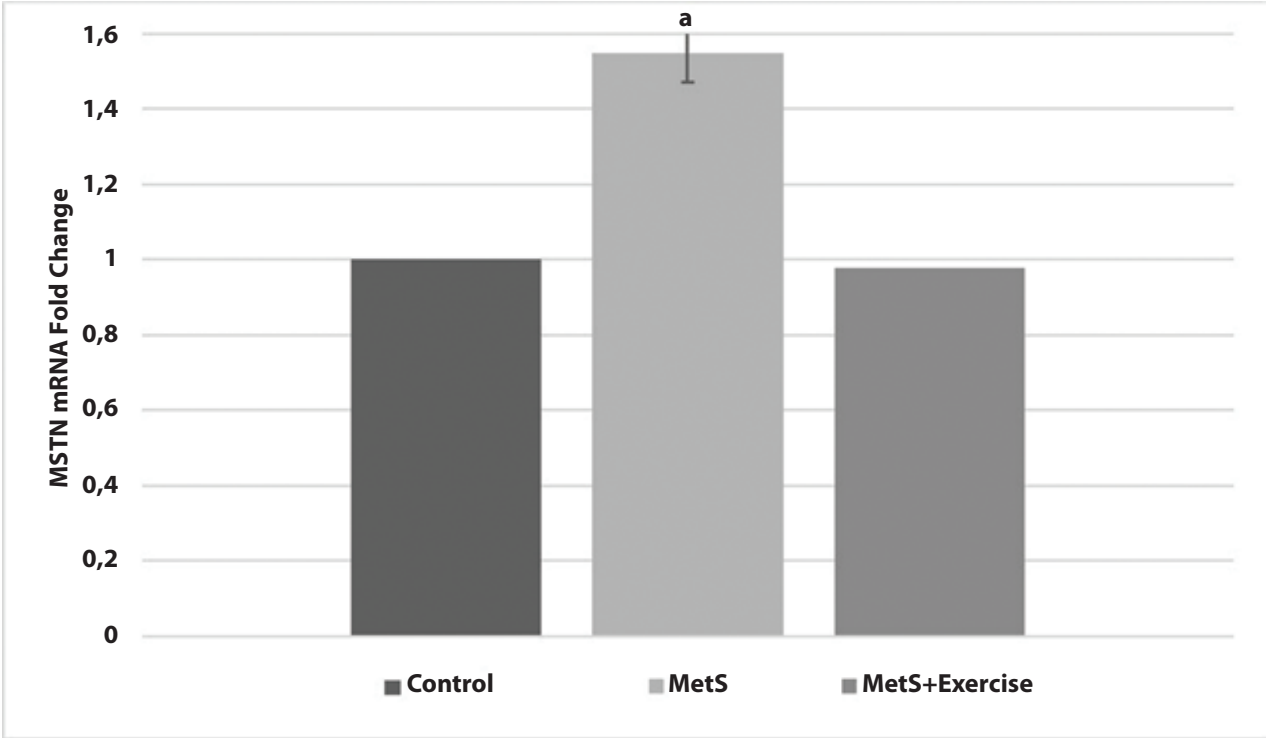

Figure 3. MSTN mRNA Fold Change.

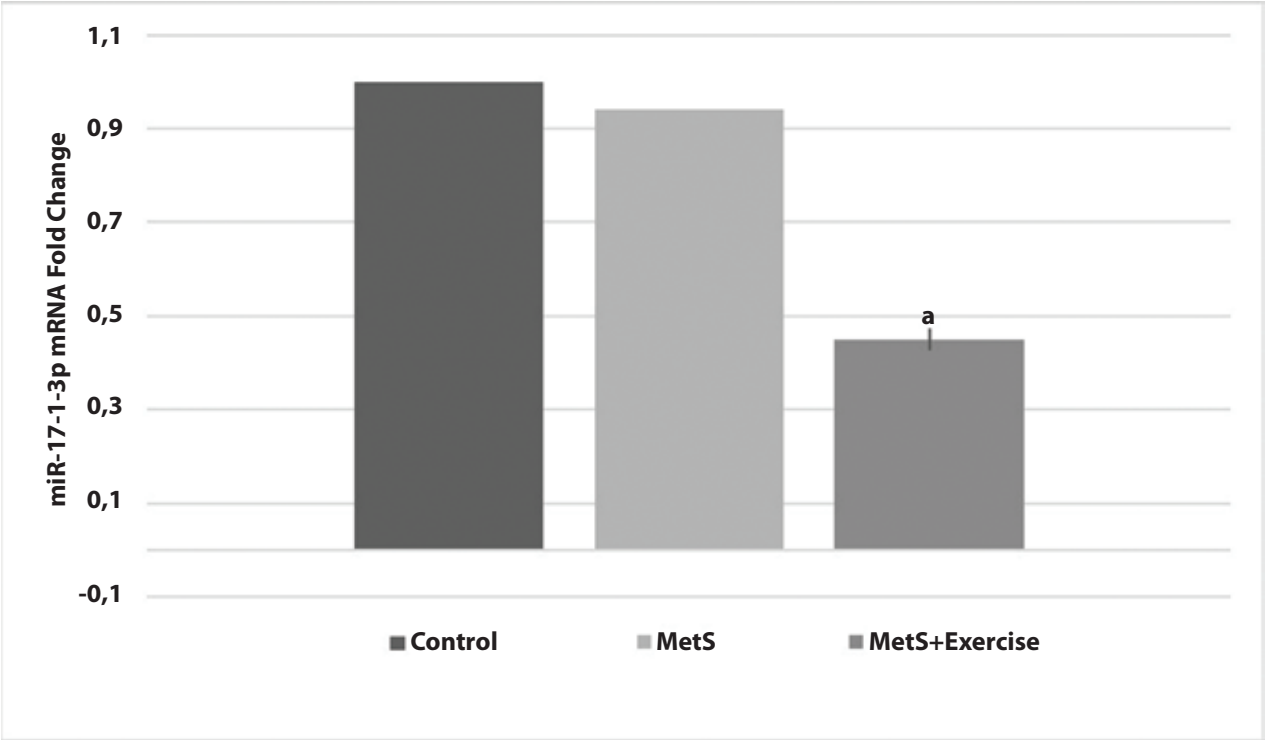

Figure 4. miR-17-1-3p Fold Change.

industry, make MetS widespread to a pandemic. In studies investigating the negative effects of MetS on molecular mechanisms, it is emphasized that MetS is associated with mitochondrial dysfunction (31). It is discussed by the scientists that exercise, which has a wide range of effects in terms of intensity, duration and frequency, can be an effective method in combating metabolic diseases. However, the molecular mechanisms underlying the tangible and visible physical positive effects of exercise have not yet been fully elucidated. To the best of our knowledge, in this study, we have associated miR-17-1-3p for the first time in the regulation of skeletal muscle mitochondrial fusion genes (Mfn1, Mfn2) and MSTN gene expressions involved in skeletal muscle cell biogenesis, in a rat MetS model.

MetS, which is a precursor of obesity, diabetes and even cardiovascular diseases, causes fluctuations of Mfn1 and Mfn2 gene expressions, which control mitochondrial fusion dynamics (5). In the study by Tyagi et al. 2018, Downregulation of Mfn1 and Mfn2 gene expressions in brain tissues of rats with MetS has been demonstrated (5). On the other hand, Durak et al. 2018 demonstrated the upregulation of Mfn1 and Mfn2 gene expressions in kidney and cardiac muscle tissues of rats with MetS (32). Changes in the regulation of mitochondrial fusion may differ in tissue specificity. In our study, although it was not statistically significant, Mfn1 and Mfn2 expressions, which were downregulated in MetS, were upregulated after six weeks of swimming exercise. This result demonstrates the therapeutic effect of exercise at the cellular level. Moreover, in parallel with our study, different studies have tried to emphasize that exercise is an important strategy to eliminate the imbalance in mitochondrial dynamics. (28,33,34).

It is supported by studies in the literature that MSTN, which is significantly released from skeletal muscle and known as a negative regulator of muscle development, is upregulated in MetS (35-38). Similar to these studies, we also showed in our study that MetS upregulates MSTN expression. The relationship between MSTN and exercise has been investigated since its regulatory effect on muscle biogenesis gained

importance. Moreover, most of the studies suggest that MSTN, whose expression is upregulated due to any disease or ageing, can be controlled with exercise therapy (38-41).

Although the control potential of miRNAs on gene expressions in metabolic pathways is known, it is not yet clearly understood. However, some members of the miR-17 family (miR-17-5p, miR-17-92, miR-17-3p) have been the subject of some research in metabolic or other diseases (42,43) and exercise interventions (17- 21,42,43), to the best of our knowledge, miR-17-1-3p was associated with exercise intervention in MetS rat model for the first time in our study. It is known that mir-17-1-3p targets the Mfn2 gene expression at a level of 87% (44). In the present study, miR-17-1-3p expression, in which MetS did not cause a significant change, was statistically significantly downregulated after six weeks of swimming exercise. Moreover, we think that, suppressed miR-17-1-3p expression is associated with upregulated Mfn1, Mfn2 and downregulated MSTN expressions in exercise intervention.

As a result; we have shown that treating MetS with exercise therapy upregulates Mfn1 and Mfn2 gene expressions, which are involved in mitochondrial fusion dynamics, and that MSTN expression, which is increased in MetS, is downregulated by exercise intervention. It is thought that, the role of MetS in impaired mitochondrial formation and muscle atrophy can be prevented by exercise therapy. It is considered that exercise regulates MSTN, Mfn1 and Mfn2 gene expressions by suppressing miR-17-1-3p. However, we do not have researchers and infrastructure to perform histochemical evaluation in this project, this could be accepted as a limitation of this study. In order to elucidate the molecular mechanisms underlying these effects, we believe that the suppression of miR17-1-3p by exercise can be explained by revealing the positive effects of exercise by regulating microRNAs.

Acknowledgement. The study was supported by Firat University Scientific Research Projects Unit (FUBAP).

Conflict of Interest. Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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